DNA Cleavage Using Artificial Hydrolases Based on Cobalt (III) Cyclen Complexes

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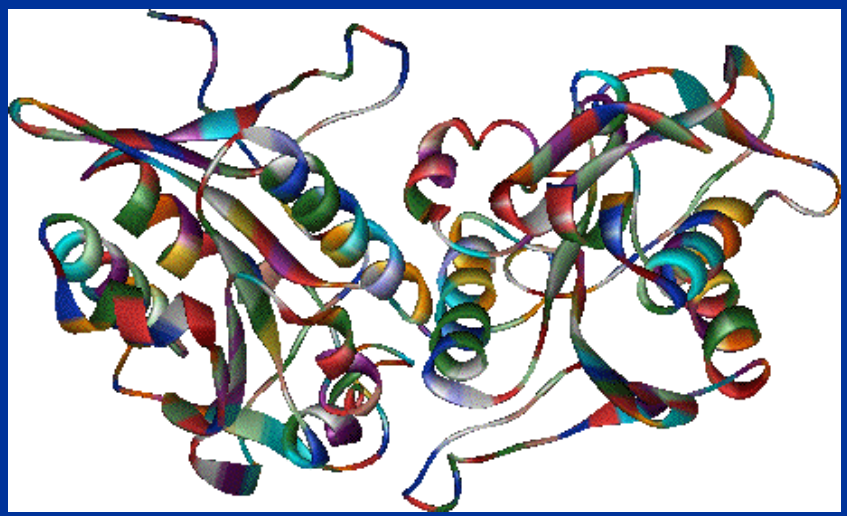

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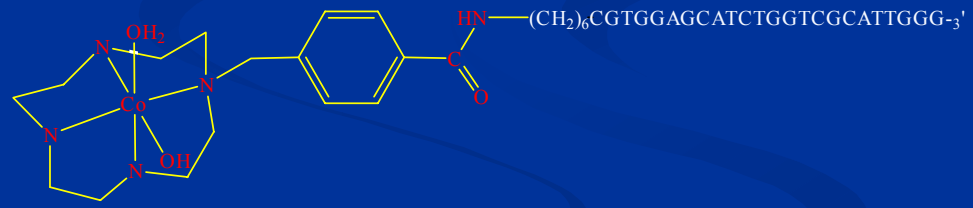

Artificial Hydrolase

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ABSTRACT

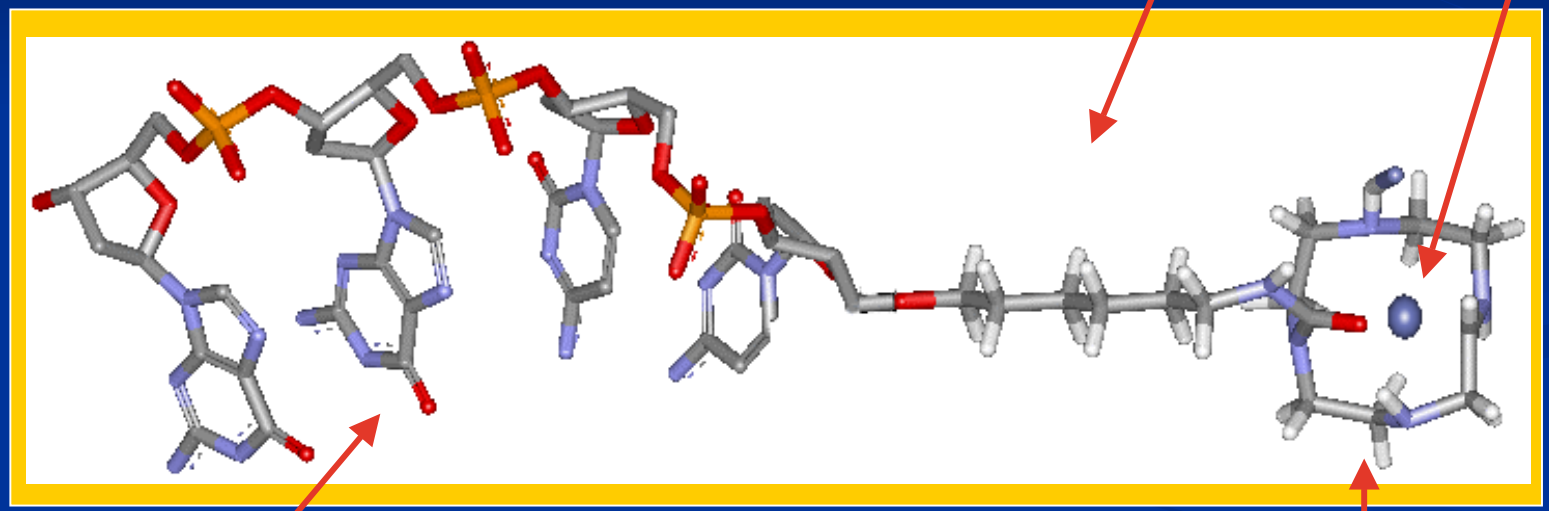
We are developing small, robust catalytic systems to efficiently degrade DNA/RNA with a high degree of specificity. Here, we describe the use of tetraazamacrocyle-chelated Co(III) complexes for the effective hydrolysis of double stranded plasmid DNA. These chelator molecules have been functionalized with a coupling group and we examine whether the modification has affected the ability of the complex to degrade DNA. Hydrolysis of DNA does appear to be slowed by the intermediate functionalization, but the eventual form of the coupled chelator-complex shows very high activity against a model pBluescript supercoiled DNA. We discuss these results, future tests, and plans for using these systems for sensitive equipment decontamination.

Introduction

Naturally occurring nucleases, while potent as native defenses against foreign DNA, are limited by their short sequence-recognition capability and temperature instability. Development of "artificial nucleases" can overcome these limitations.

We seek to develop strongly binding artificial nucleases, with enhanced sequence recognition/ specificity and stability and to determine their suitability for anti-bacterial and anti-viral applications. These systems will be developed specifically to disrupt or destroy viruses and/or bacteria either through direct contact of the nucleases with the target genetic material or by delivering the nucleases through the protective envelopes of microorganisms using suitable carriers. The novel nucleases will be tested as decontamination agents.

Components of Our System

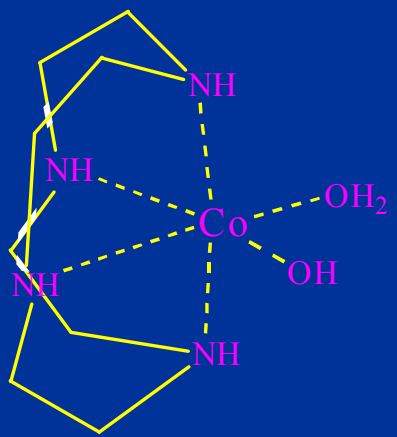


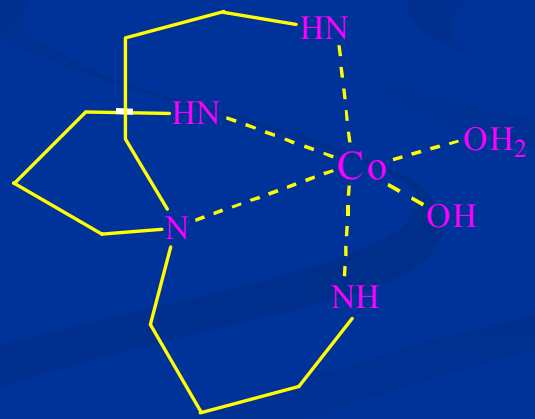
Co(III)-Chelator Characteristics

- Among the fastest in hydrolysis of phosphodiester systems
- Kinetically inert, i.e., no turnover at RT
- "Promotes" hydrolysis, rather than "catalytic" at RT
- Inertness to help supress viral growth

How Fast?

	Cyclen	TRPN
BNPP	$4.6 \times 10^{-3}\ s^{-1}$	$2.5 \times 10^{-2}\ s^{-1}$
DNA	$2 \times 10^{-4}\ s^{-1}$	$5 \times 10^{-5}\ s^{-1}$

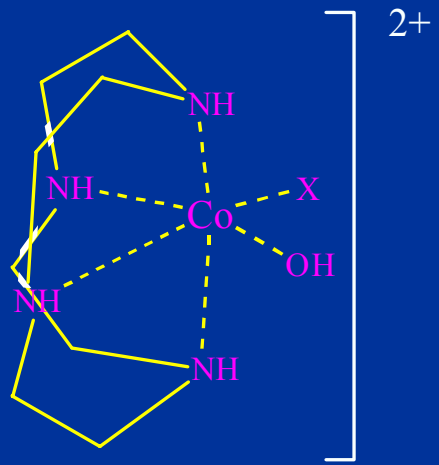




Kinetic Inertness

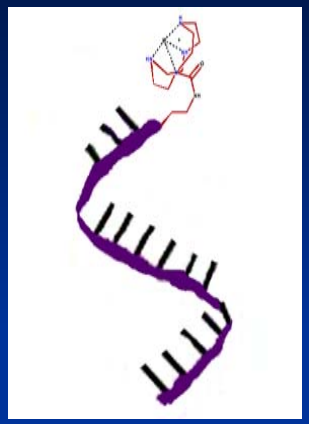
Substitutional Inertness:

$L_nM\text{-}X + Y \rightleftharpoons L_nM\text{-}Y + X$ Ligand exchange is slow.



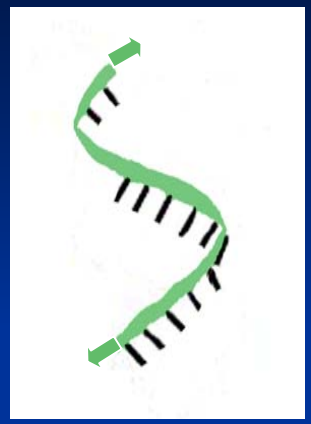
Example of a kinetically inert chelator-Co(III) complex

Anti-Sense Paradigm

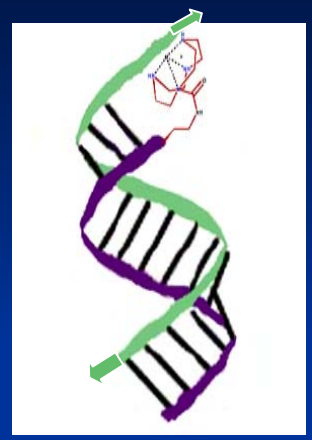


ODN probe + catalyst

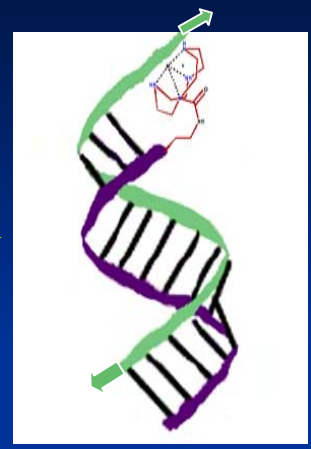
+



RNA

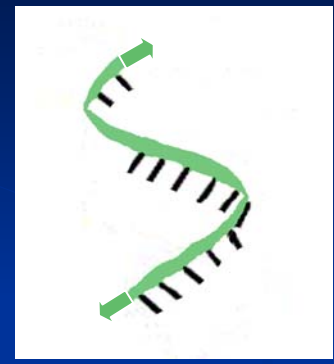


probe + target RNA + catalyst



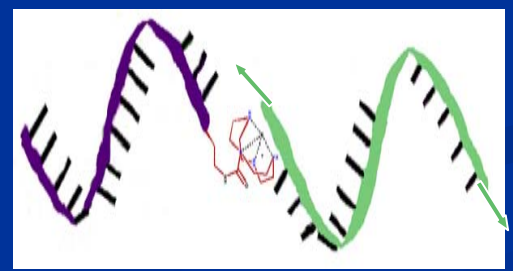
probe + target RNA fragment + catalyst

+



RNA fragment

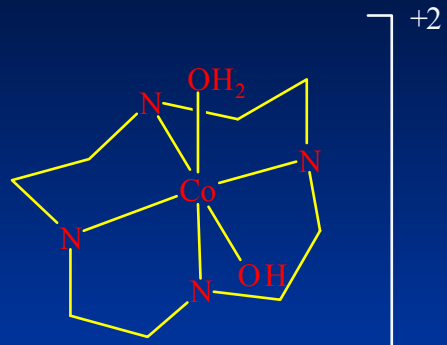
Hydrolysis



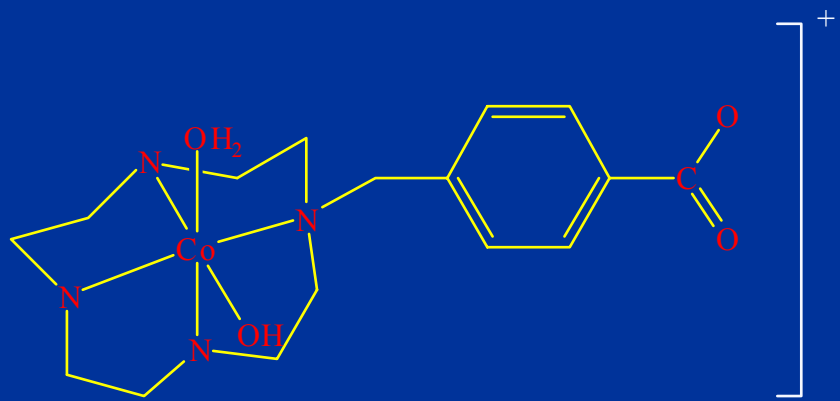
probe + target RNA + catalyst

kinetic inertness used for added steric blockade advantage

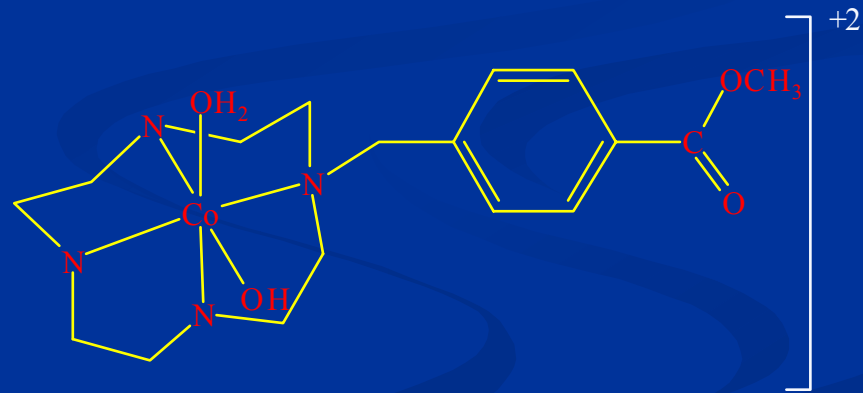
CoCyclen and Its Functionalized Forms



CoCyclen

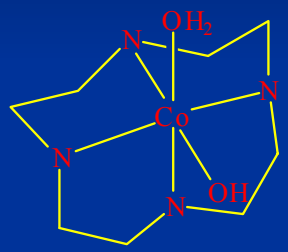


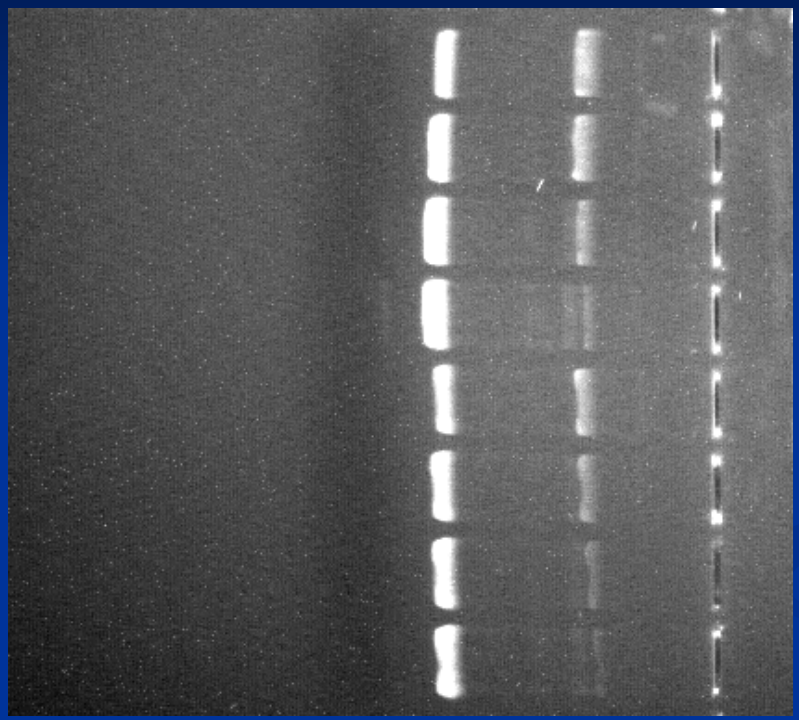
CoCyclenmba



CoCyclenmmb

CoCyclen Hydrolysis of dsDNA





5mM Cocyc + DNA 37˚C

2.5mM Cocyc + DNA 37˚C

1.25mM Cocyc + DNA 37˚C

DNA 37˚C

H
E
A
T
80˚C, 5 min
25˚C, 5min

5mM Cocyc + DNA 37˚C

2.5mM Cocyc + DNA 37˚C

1.25mM Cocyc + DNA 37˚C

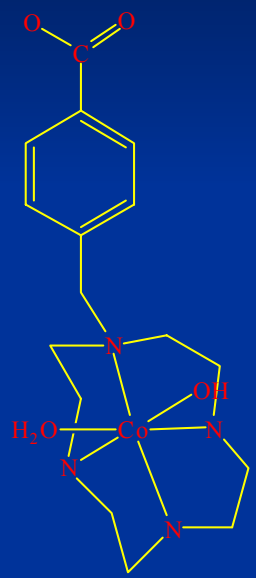
DNA 37˚C

uncut dsDNA

nicked DNA

Unmodified chelator hydrolysis of dsDNA at 37°C for 2 h.
Increasing nicked DNA as a function of Co(III) concentration.

CoCyclenmba Hydrolysis of dsDNA



pH 10 control

pH 10

pH 8 control

pH 8

pH 7 control

pH 7

Nickase control

Nickase

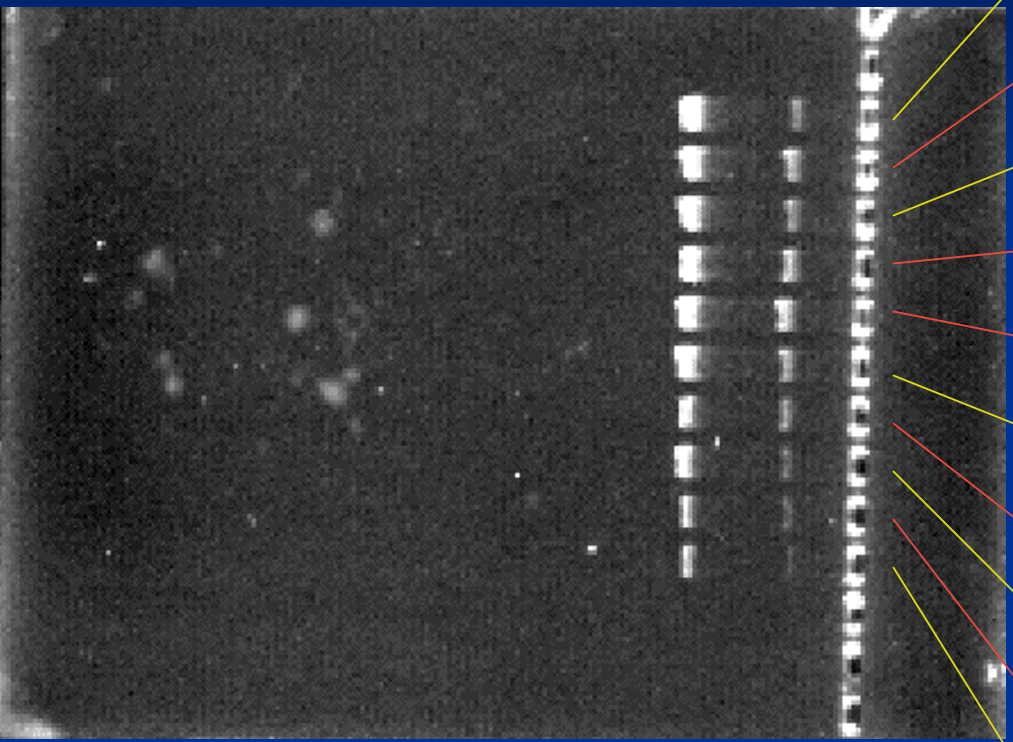
pH 7 fastest for functionalized hydrolysis center. Possible slight increase at pH 10.

Cocyclenmba Results

Lane	Supercoiled	Linear	Nicked	Comments
8	93%	3%	4%	pH 10 cntl
7	84%	5%	12%	pH 10
6	92%	5%	3%	pH 8 cntl
5	87%	5%	9%	ph8
4	90%	4%	5%	pH 7 cntl
3	72%	3%	25%	pH 7
2	93%	7%	0%	nickase cntl
1	10%	4%	87%	nickase

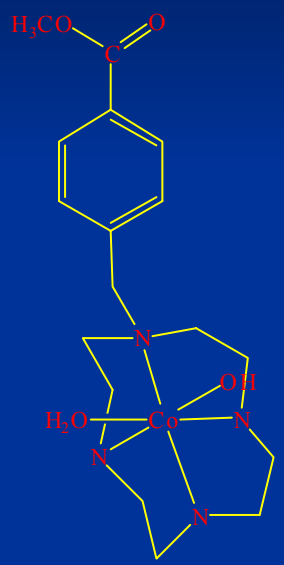
Cocyclenmba is most active at pH 7 with about 25% nicked population.

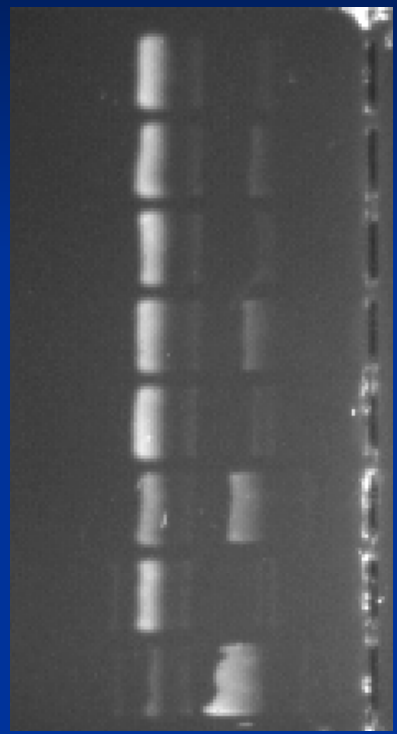
CoCyclenmba Concentration-Dependence



Lane	[cocycmba]	[DNA]
1.	1.7 mM (ctrl)	0.139 mM bp
2.	1.7 mM	0.139 mM bp
3.	3.4 mM (ctrl)	0.139 mM bp
4.	3.4 mM	0.139 mM bp
5.	5 mM	0.139 mM bp
6.	5 mM (ctrl)	0.139 mM bp
7.	5 mM	0.0696 mM bp
8.	5 mM (ctrl)	0.0696 mM bp
9.	5 mM	0.0348 mM bp
10.	5 mM (ctrl)	0.0348mM bp

Cocyclenmmb Hydrolysis of dsDNA





pH 10 control

pH 10

pH 8 control

pH 8

pH 7 control

pH 7

Nickase control

Nickase

pH 7 fastest for "coupled" form of the hydrolysis center--about 50% of nickase activity.

Cocyclenmme Results

Lane	Supercoiled	Linear	Nicked	Comments
8	93%	3%	4%	pH 10 cntl
7	89%	4%	7%	pH 10
6	90%	5%	5%	pH 8 cntl
5	81%	4%	15%	ph8
4	91%	4%	5%	pH 7 cntl
3	54%	2%	43%	pH 7
2	94%	2%	4%	nickase cntl
1	9%	3%	88%	nickase

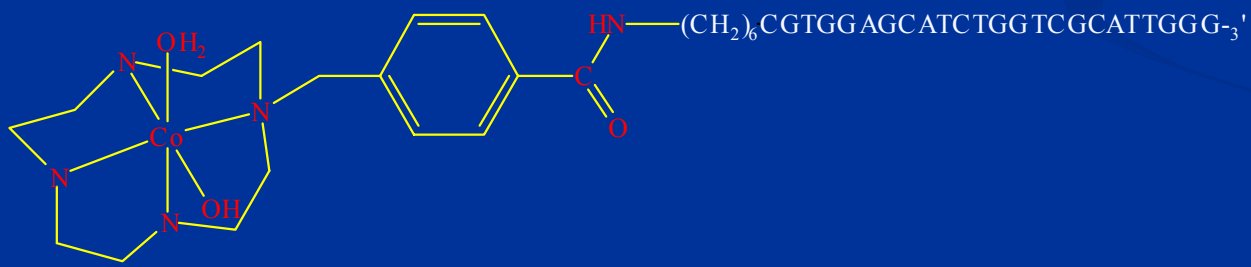
Cocyclenmme is most active at pH 7 with 43% nicked population--almost half of the nickase rate.

Next Steps

Sequence-Specific Cut of a ssDNA

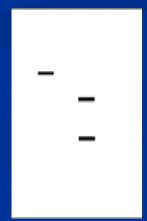
5' GCGCTGACCC GCACCTCGTA GACCAGCGTA
ACCCAGTGGT CGTTTAGCGC GACAATCGCC
CGGGTAATTC AAGACAGAGC CGCGCAGACG
CAGACCGACC 3'

100-mer target ssDNA (substrate)



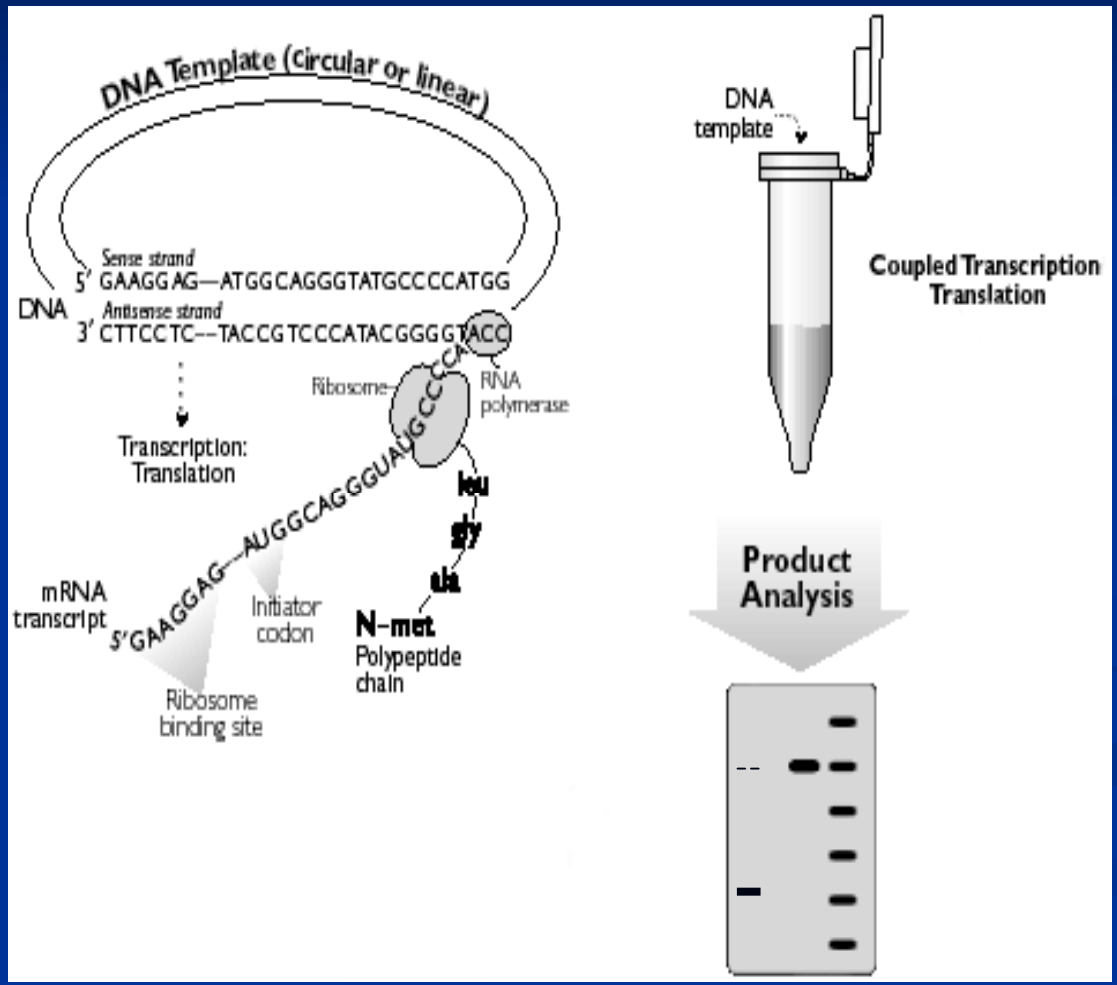
24-mer CoCyclenmba complex

2 products around 34- and 66-bases long



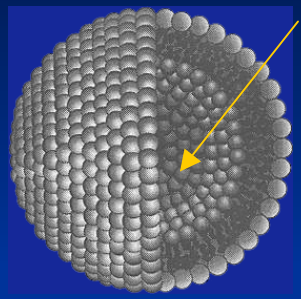
Cell-Free Demonstration

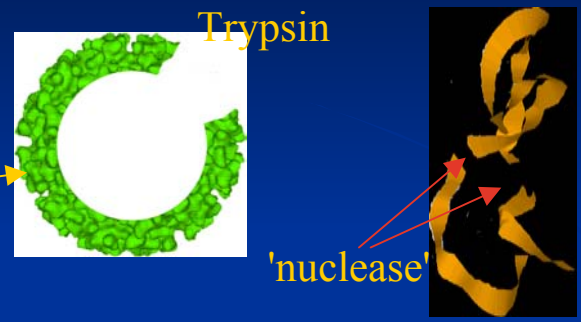
Inhibition of a transcription/translation system

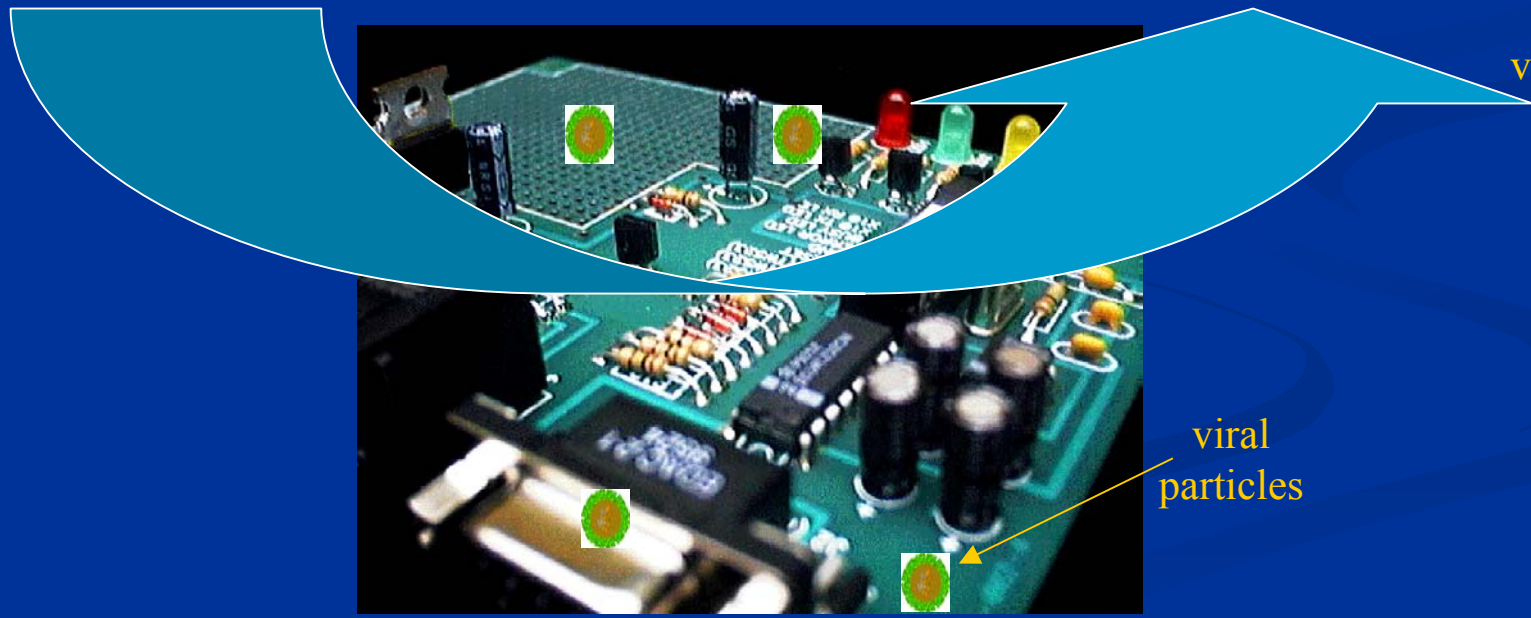


Decontamination

Package into delivery platform, e.g., fusion-enabled vesicles:

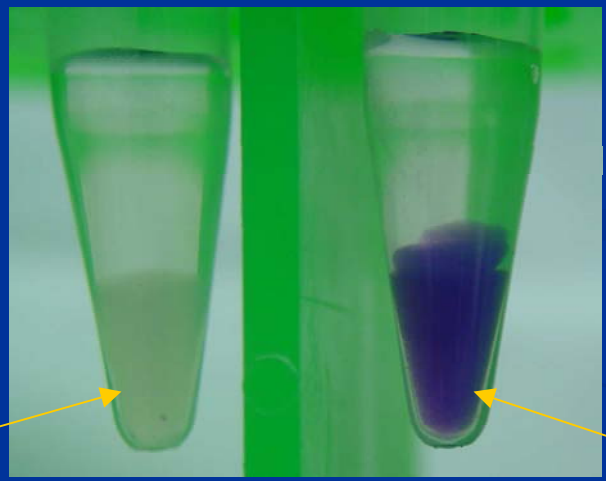






Coupled to Beads

We have also coupled the functionalized complex to solid-supports, such as agarose beads. These can either serve as filtration elements or as a way to produce ssDNA-chelator complexes.



Control agarose beads

Cocycmba-coupled agarose beads

Conclusion

We have synthesized a Co(III)-chelator complex with a coupling functionality and tested the system against dsDNA, using the amount of nicked dsDNA produced as a measure of activity.

The functionalized complex was active against pBluescript supercoiled DNA with the methyl-ester most active at close to 50% activity of a control nickase enzyme.

Our next steps are to couple the system to a ss-oligo "primer" for sequence-specific cutting and then to a demonstration of cell-free inhibition of protein production via nucleic acid interdiction.

This work was partially supported by ONR and associated DoD organizations.